A SIMPLE TECHNIQUE FOR MEASURING THE M² PARAMETER, OPTICAL FOCUS, ASTIGMATISM AND ELLIPTICITY OF THE LIGHT EXITING AN OBJECTIVE LENS IN A LASER SCANNING MICROSCOPE

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KEY WORDS: Beam measurement, M-squared, ellipticity, confocal, multi-photon

We report a method for measuring the optical quality of the post-objective laser radiation in a laser scanning microscope. We use a microscopic-scale knife-edge in the form of a simple transmission electron microscopy grid attached to a glass microscope slide and a light-collecting optical fibre and photodiode underneath the specimen. By scanning the laser spot from a reflective to a transmitting part of the grid, an error function measuring the beam can be obtained and by repeating this with the knife-edge at different axial positions relative to the beam waist, the M² parameter of the post-objective laser beam can be obtained, together with measurements of the size of the optical focus, the ellipticity and the astigmatism of the laser beam exiting the objective lens. This measurement is useful not only for measuring the size of the optical focus, but also for evaluating the beam divergence and thus evaluating how much of the full numerical aperture of the lens is used in practice, which impacts the image quality in confocal and multi-photon microscopy.

Figure 1. Experimental data of the evolution of the beam radius for a 5x/0.15 numerical aperture lens used with 488nm radiation in a laser scanning confocal microscope. The data points (crosses) are presented with the calculated M²=1.68 (solid line) and ideal M²=1 (dashed line).

We will present details of the measurement technique, as well as data of beam divergence, ellipticity, astigmatism and M² parameters obtained with very high (1.3) and low (0.15, 0.7) numerical aperture lenses and lasers commonly used in confocal and multi-photon laser scanning microscopy.